



A field method of determining NH_4^+ and NO_3^- uptake kinetics in intact roots: Effects of CO_2 enrichment on trees and crop species

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Abstract

Models describing plant and ecosystem N cycles require an accurate assessment of root physiological uptake capacity for NH_4^+ and NO_3^- under field conditions. Traditionally, rates of ion uptake in field-grown plants are determined by using excised root segments incubated for a short period in an assay solution containing N either as a radioactive or stable isotope tracer (e.g., $^{36}\text{ClO}_3$ as a NH_4^+ analogue, $^{14}\text{CH}_3\text{NH}_3$ as an NO_3^- analogue or $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$). Although reliable, this method has several drawbacks. For example, in addition to radioactive safety issues, purchase and analysis of radioactive and stable isotopes is relatively expensive and can be a major limitation. More importantly, because excision effectively interrupts exchange of compounds between root and shoot (e.g., carbohydrate supply to root and N transport to shoot), the assay must be conducted quickly to avoid such complications. Here we present a novel field method for simultaneous measurements of NH_4^+ and NO_3^- uptake kinetics in intact root systems. The application of this method is demonstrated using two tree species; red maple (*Acer rubrum*) and sugar maple (*Acer saccharum*) and two crop species soybean (*Glycine max*) and sorghum (*Sorghum bicolor*). Plants were grown in open-top chambers at either ambient or elevated levels of atmospheric CO_2 at two separate US national sites involved in CO_2 research. Absolute values of net uptake rates and the kinetic parameters determined by our method were found to be in agreement with the literature reports. Roots of the crop species exhibited a greater uptake capacity for both N forms relative to tree species. Elevated CO_2 did not significantly affect kinetics of N uptake in species tested except in red maple where it increased root uptake capacity, V_{\max} , for NH_4^+ . The application, reliability, advantages and disadvantages of the method are discussed in detail.

Introduction

Soil physical and chemical properties such as bulk solution concentration, diffusion and mass flow determine the availability of mineral nutrients at the root surface (Nye and Tinker, 1977). However, the rate of ion entry into the plant is regulated by root physiological uptake kinetics (Barber, 1984; Chapin, 1980; Clarkson, 1985; Schenk, 1996) which plays an important role in determining overall plant nutrient status. Root nutrient uptake kinetics vary widely

among species and genotypes and this variation may explain differences in competitive ability in nutrient acquisition among co-occurring species (Chapin et al., 1986; Lambers and Poorter, 1992) influencing the dynamics of ecosystem nutrient cycling. Despite its significant role in regulating plant and ecosystem nutrient dynamics, measurement of root uptake kinetics in field-grown plants remains a difficult task.

Currently, the excised root technique (Epstein, 1972) is the most common field method for measuring root uptake kinetics. Briefly, in this method fine active roots are obtained from soil cores or other soil excavation techniques, washed and cut into 5 to 10 mm

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long segments and are further divided into subsamples. Each subsample is then placed in a buffered uptake solution for a period of up to two hours. The uptake solution contains a radioactive or stable isotope tracer of the nutrient of interest (e.g., ^{32}P and ^{15}N are often used to measure P and N uptake). Rates of uptake in the saturable ranges of the substrate (labeled ion) concentration are determined by analyzing tracer accumulation in the tissue. Often a minimum of five or six data points are required to construct the uptake rate vs. concentration curve for calculation of kinetic parameters V_{\max} and K_m .

Although excised root techniques can produce relatively reliable estimates of root physiological uptake kinetics, their application in field research is subject to a number of limitations. For example, since excised roots are cut from the endogenous carbohydrate supplies, uptake measurements have to be conducted rapidly following the excision to avoid carbohydrate exhaustion in the root tissues. In most cases, this problem is minimized by exogenous addition of carbohydrates (e.g., glucose or sucrose) into the uptake solution. However, as excised roots can readily absorb and metabolize soluble sugars (Bryce and Ap Reese, 1985), results may be compromised particularly in studies where the treatment is expected to affect nutrient uptake via root carbohydrate status.

Excised root experiments also require use of isotopic tracers as a labeling tool. There are several drawbacks associated with such a requirement. First, all radioactive tracers such as ^{13}N and ^{32}P pose a potential health hazard. Therefore, their use and waste disposal requires special training and care. Second, for N uptake studies, only a few radio tracers are available, but each offer a unique disadvantage. For example, ^{13}N has a half life of roughly 10 min. which limits its utility for field experiments. On the other hand, the use of analogues such as ClO_3 (synthesized with ^{36}Cl) for NO_3^- and CH_3NH_3 (labeled with ^{14}C) for NH_4^+ uptake studies have recently been criticized for not reliably reflecting the uptake behavior of NO_3^- and NH_4^+ (Jackson and Bloom, 1994; Kosola and Bloom, 1994, 1996; Siddiqi et al., 1992). More recently, ^{15}N is widely used as a tracer in N uptake studies. ^{15}N has very little or no hazard potential and as such is an attractive tracer of N. Nevertheless, because tissue ^{15}N analysis is fairly expensive its utility particularly in large-scale experiments may be cost prohibitive.

Here we describe a novel method of simultaneously determining kinetics of NH_4^+ and NO_3^- uptake

of intact root systems in the field. The application of the method is demonstrated using two tree and two crop species. Since the method uses intact root systems, the possibility of carbohydrate exhaustion is avoided. Furthermore, because NH_4^+ and NO_3^- uptake rates are calculated from the depletion of these ions, (1) changes in the solution concentration can be analyzed by a range of conventional analytical methods for NH_4^+ and NO and (2) there is no requirement for use of tracers. We are particularly interested in examining how projected doubling of atmospheric CO_2 concentration will affect root uptake capacities of these inorganic N forms in intact roots of field grown plants. The knowledge of species differences in root N uptake kinetics and their relative responses to increases in atmospheric CO_2 is critical in predicting ecosystem productivity and potential changes in species composition. To date, there is little information about the potential impact of high CO_2 on kinetics of root N uptake in tree species (BassiriRad et al., 1997a, b) and to our knowledge this is the first report of such measurements in intact field-grown plants.

Materials and methods

Growth conditions

This work was conducted in the summer of 1996. Two national sites involved in CO_2 research were used: (1) Oak Ridge National Laboratory's Global Change Field Research Facility on the National Environmental Research Park in Oak Ridge, TN and (2) USDA-ARS National Soil Dynamics Laboratory in Auburn, AL. The work involving the tree species was conducted in twelve open-top chambers (OTC) at Oak Ridge. These OTCs were constructed (Rogers et al., 1983) at the facility on soils classified as Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudult) with moderate-to-medium granular structure and medium internal drainage. The chambers were 3.0 m in diameter and 2.4 m high. An additional 1.2 m panel was installed at the beginning of the third growing season (1996) to accommodate the height growth of the seedlings. The chambers were modified to operate at either ambient temperature or 4 degree C above ambient, in combination with ambient or elevated (+30 Pa) atmospheric CO_2 partial pressure (Norby et al., 1997). A randomized complete block design was used with four treatments (the factorial combinations of two CO_2 levels with two temperature regimes) in each of three

blocks. In the current investigation only the ambient temperature chambers were used.

One-year-old red maple (*Acer rubrum* L.) and sugar maple (*Acer saccharum* Marsh.) seedlings from a commercial nursery were planted directly into the soil within the chambers in spring, 1994. Additional seedlings (not used in the current study) were planted in spring, 1995, for a total of 10 plants of each species per chamber. The sides and tops of the OTCs were covered with 73% shade cloth to avoid unnatural levels of side light to these shade-tolerant maple seedlings. When roots were sampled in this experiment in June, 1996, the average height and basal diameter across the treatments were 2.1 m and 20 mm for red maple and 2.4 m and 18 mm for sugar maple. Trees in elevated CO₂ had an estimated aboveground dry mass, based on basal area times height, that was 76% greater in red maple and 23% higher in sugar maple compared to trees in ambient CO₂.

The experiments on crop species was also conducted in OTCs (Rogers et al., 1983) that were almost identical to those used at Oak Ridge and were part of an ongoing CO₂ experiment initiated in June 1992. Carbon dioxide levels inside the chambers were set at partial pressures of ambient or ambient plus 36 Pa. The experimental site was a soil bin (2 m deep, 6 m wide, and 76 m long) which was uniformly filled with surface soil of a Blanton loamy sand (loamy, siliceous, thermic Grossarenic Paleudult) that had been continuously fallow for over 25 years (Batchelor, 1984). A more detailed description of the soil status prior to initiation of the study, fertilizer and lime amendments during the study, and subsequent soil analysis results have been reported previously (Reeves et al., 1994). All experimental plots were managed under no-till conditions since 1992.

Crop species tested here were soybean (*Glycine max* (L.) Merr.; 'Stonewall') and grain sorghum (*Sorghum bicolor* (L.) Moench.; 'Savanna 5'). Plants were grown from seed to maturity in OTCs at two CO₂ levels. Seeds were sown on 9 May in 1996. Soybean seeds were inoculated with commercial *Rhizobium* (Nitragin Co.) prior to planting. Plants were thinned for uniformity to a final density of 30 plants m⁻² for soybean and 26 plants m⁻² for sorghum. To ensure adequate plant establishment, fertilizer N was broadcast at a rate of 34 kg N ha⁻¹ to both the grain sorghum and the soybean shortly after planting. In the grain sorghum, an additional 67 kg N ha⁻¹ was applied 30 days after planting. Weed control during the growing season was done by hand. In the off season, weed con-

trol was done both by hand and by use of glyphosate (N-[phosphonomethyl] glycine). At the time of uptake studies, plants were near the end of their vegetative growth.

The Auburn experiment was a split-plot design with three replicate blocks (6 m by 25 m). Whole plot treatments (plant species) were randomly assigned to half of each block. Subplot treatments (CO₂ levels) were randomly assigned to two open top chambers (3 m dia.) within each whole plot. Nutrient uptake experiments on these species were also performed in June of 1996 within a week of the tree species.

Root NH₄⁺ and NO₃⁻ uptake kinetics

Experimental procedures for uptake experiments were similar for both sites. Net influx of NH₄⁺ and NO₃⁻ for the target roots were determined by the depletion technique. Higher order laterals, containing a large number of fine active roots, were carefully dug out to a shallow soil. In all cases, sufficient suitable roots were present in the top 5 cm. Excavation of fine roots proceeded rapidly and cautiously until enough root material was exposed. Fine roots less than 2 mm in diameter with either white or light brown color were considered active in N uptake. Soil particles were carefully and thoroughly washed off the fine intact roots with distilled water making sure to avoid any physical damage. Immediately after rinsing, fine roots, still attached to the plant, were placed into a 2 mL microfuge tube containing 1.5 mL solution of NH₄NO₃ at one of the following concentrations: 25, 50, 75, 100, 150 and 200 μM. Uptake solutions also contained a phosphate buffer composed of 1 mM, KH₂PO₄ and 0.05 mM, K₂HPO₄ which maintained the pH at 6.0. More recent work in our lab indicates that rates of NH₄⁺ and NO₃⁻ uptake do not differ significantly in the presence or absence of this phosphate buffer. Therefore, the use of phosphate buffer is not critical. The solutions were well aerated prior to uptake assay.

Roughly 5 to 10 mg of root dry weight were used in crop species and 10 to 20 mg in tree species. The amount of roots placed in each uptake tube was determined empirically. The amount of root necessary for this method may vary significantly for different species and functional groups. The ratio of root to solution volume has to be adjusted so that there is a detectable depletion during the uptake period, but to avoid a complete exhaustion of NH₄⁺ or NO₃⁻ concentration prior to the completion of the incubation period. After immersing the roots into the uptake solu-

tion, the microfuge tubes were covered tightly with a piece of parafilm to reduce evaporation. All other exposed root parts were covered with wet paper towels throughout the uptake period. The incubation period lasted thirty to sixty minutes at which time the uptake assay was terminated by removing the roots from individual assay solutions. At that point, the portion of roots immersed in the assay solution was cut from its lateral and oven dried to be used in the uptake rate calculations. The microfuge tubes containing the N depleted assay solutions were capped tightly and frozen in liquid nitrogen until they were analyzed for NH_4^+ and NO_3^- .

Concentrations of NH_4^+ and NO_3^- were simultaneously determined on an automated HPLC system modified after Goyal and Huffaker (1986). Briefly, The HPLC system used consisted of the following: two LC pumps, an SIL-10A autosampler, an SPD-10A variable wavelength UV detector, an RF-10A^{XL} fluorescence detector, and an SCL-10A system controller all from Shimadzu Scientific Instruments (Columbia, MD), a home-made heat reaction coil, a Valco (Houston, TX) electrically actuated nine port eight position stream selection valve, and a manual three port two position selection valve from Upchurch Scientific (Oak Harbor, WA). The autosampler was equipped with a 50 μL sample loop. Data collection and analysis from the HPLC system was performed using a software, CLASS-VP, which was also provided by Shimadzu. From each sample only 10 μL volume was needed to analyze for both NH_4^+ and NO_3^- concentrations. NO_3^- was isocratically eluted from a strong anion exchange Zorbax column (4.6 \times 12.5 mm, 5 μm , Mac-Mod analytical, Chads Ford, PA) using 50 mM phosphate buffer (pH 3.0) at a flow rate of 1 mL/min. NO_3^- was detected by UV at 210 nm. The same sample was then derivatized the on line addition (via a PEEKTM tee-connector) of a 100 mM phosphate buffer (pH 7.0) containing 3.5 mM o-phthaldialdehyde (OPA) and 5 mM 2-mercapthoethanol flowing at a rate of 1.5 mL/min. This reaction was carried out in a 3 meter coil of a microbore Teflon tubing maintained at 63 °C. The OPA-derivitized NH_4^+ was detected by fluorescence using emission and excitation wavelengths set at 410 and 470 nm, respectively.

The detection limit of this system for either NH_4^+ or NO_3^- is in the 10^{-9} M ranges. Because water uptake by roots will contribute to changes in NH_4^+ and NO_3^- concentration, this quantity was measured for each tube and taken into account in net uptake calculations.

Net uptake rates ($\mu\text{mol g}^{-1}\text{dw h}^{-1}$) vs. concentration data were fitted to a Michaelis-Menten function (based on a Hanes plot transformation described in Price and Stevens, 1989) to estimate V_{max} and K_m .

Statistics

The results of each experiment were analyzed separately. Two-way Analysis of Variance (ANOVA) was used to test for the main effect of CO_2 on derived variables (K_m and V_{max}) for NH_4^+ and NO_3^- uptake. Values represent means calculated from three replications. Within treatments, each replicate represents a different open-top chamber. Data were tested for normal distribution and were log or square transformed as necessary. Differences were considered significant at $P < 0.05$.

Results

Figure 1 shows representative net uptake rates of both N forms in the concentration ranges of 0 to 200 μM . All species exhibited a detectable net uptake of both NH_4^+ and NO_3^- in this range. Although roots of all species were able to absorb both N forms, NH_4^+ was always taken up at a greater rate (Figure 1). In fact, for a given species and a given external concentration, net NH_4^+ uptake rate was always greater (often two fold) than net NO_3^- uptake (Figure 1). There was no significant effect of CO_2 on species preference for N form however, the relative preference for NO_3^- compared to NH_4^+ was substantially higher in crop species than in tree saplings (Figures 1 and 2). When rates of net NO_3^- uptake were calculated as a proportion of total N uptake and averaged over both CO_2 treatments we found that in soybean and sorghum an average of 40% of N was taken up as NO_3^- whereas this proportion averaged for the tree species accounted for only 28% of total N uptake. The preference for NH_4^+ compared to NO_3^- is also illustrated in Figure 2 where absolute V_{max} values for NH_4^+ are substantially greater than NO_3^- in all species.

A two-way analysis of variance, performed separately for each experiment, indicated that CO_2 enrichment did not significantly affect V_{max} values of NH_4^+ and NO_3^- in all species tested. While tree species exhibited a significant CO_2 by species interaction for maximum NH_4^+ uptake capacity, there was no significant interaction term for crop species for either N form (Table 1). When each species is analyzed separately

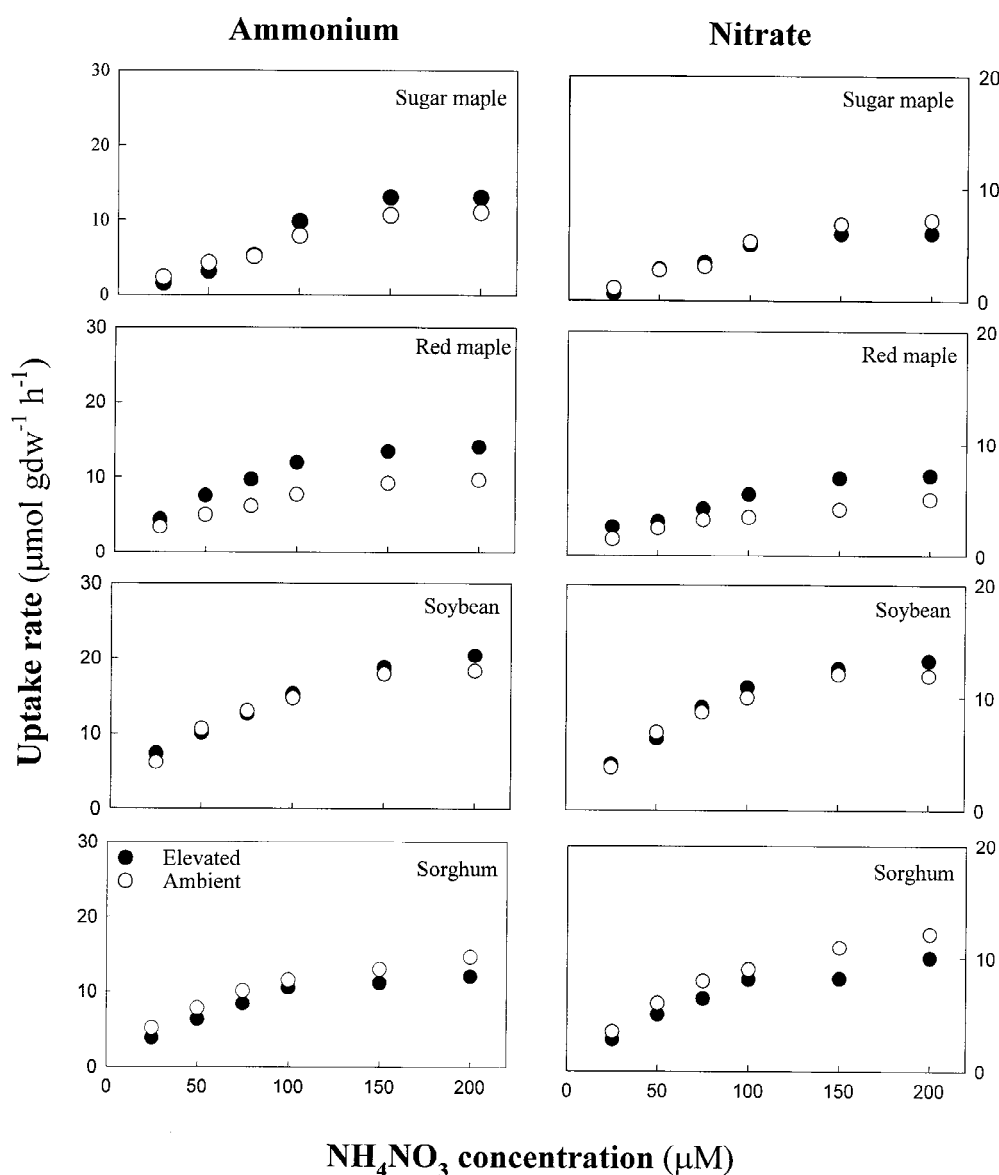


Figure 1. Root net uptake rate of NH_4^+ and NO_3^- from a wide range of external concentration of NH_4NO_3 for representative plants of two tree (red and sugar maple) and two crop species (soybean and sorghum) at ambient and elevated CO_2 levels. Rates are measured on intact field grown roots and are expressed on gram dry weight basis. Note the differences in scales of the Y axes between NH_4^+ and NO_3^- .

using a one-way ANOVA with CO_2 as the main factor, we found that CO_2 had no significant effects on either NH_4^+ or NO_3^- uptake capacity except in red maple (Figure 2). In this species, high CO_2 significantly increased V_{max} for NH_4^+ , but not for NO_3^- indicating that the significant interaction term observed in Table 1 was largely due to a positive V_{max} response of red

maple to CO_2 enrichment. There was no significant CO_2 effect K_m for either N form (data not shown).

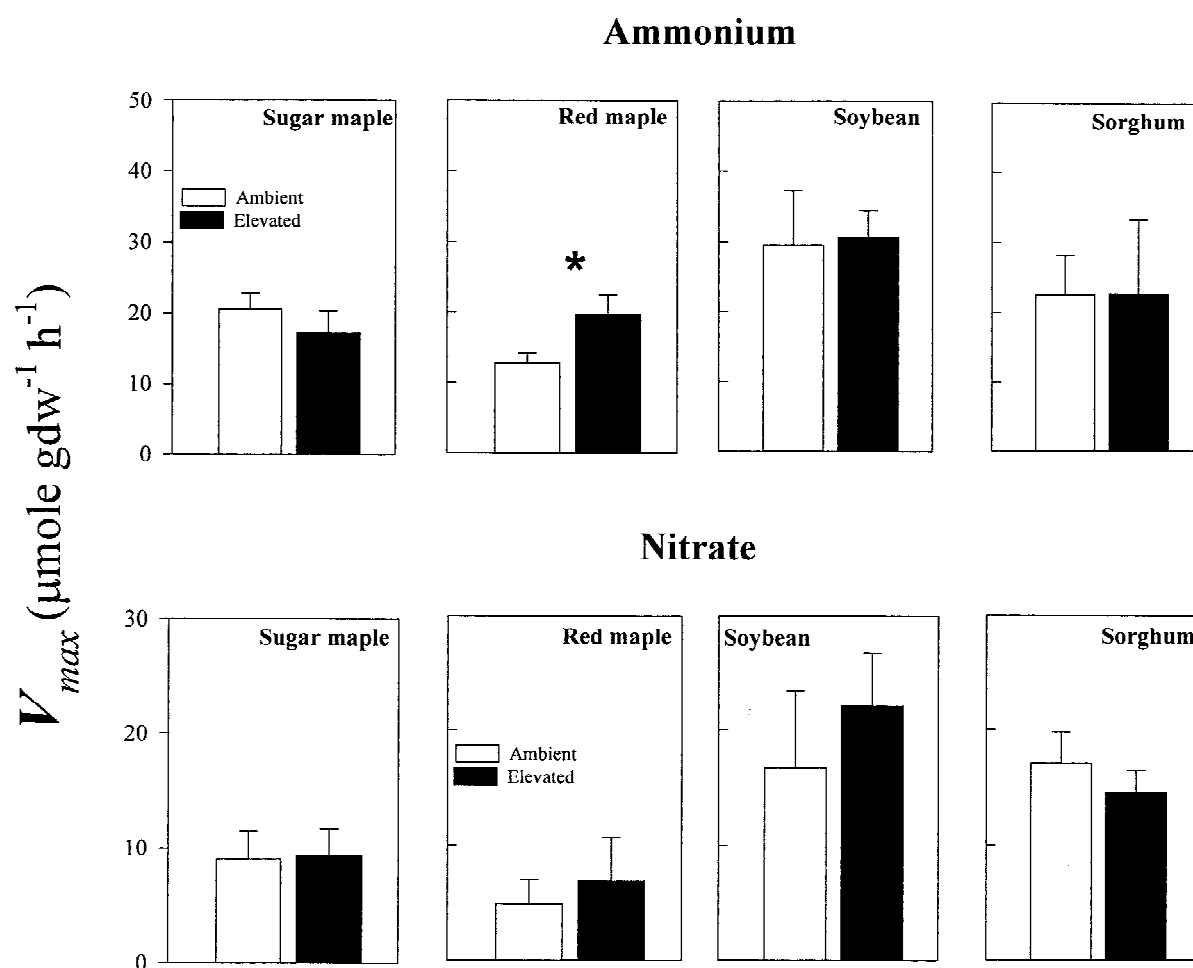


Figure 2. Maximum net uptake rate, V_{max} , of NH_4^+ and NO_3^- by intact roots of red maple, sugar maple, soybean and sorghum grown under field conditions at either ambient or elevated CO_2 levels. Each bar represents a mean of three replicates for each species. Note the differences in scales between NH_4^+ and NO_3^- . The CO_2 effect for each species is analyzed separately using a one-way ANOVA and significant differences are denoted by $*$ = $P < 0.05$.

Discussion

Reliability, advantages and disadvantages of the method

Numerous laboratory studies have previously used the depletion technique to evaluate root NO_3^- and NH_4^+ uptake kinetics, but we are not aware of any studies that have applied this approach for an *in situ* determination of N uptake kinetics. Rennenberg and colleagues described an elegant field method for determining *in situ* net uptake rates of NH_4^+ and NO_3^- in spruce and beech trees (Gessler et al., 1998; Rennenberg et al., 1996). Although their method is similar to ours in terms of using intact roots, they determined net

uptake rates of these ions at only a single external concentration of NH_4^+ and NO_3^- .

There are strong reasons why the method described here produces reliable information about an important root characteristic that is critical for a better understanding of the N dynamics in natural and managed ecosystems. The absolute values of net NH_4^+ and NO_3^- uptake rates as well as V_{max} values for both N forms reported here are qualitatively and quantitatively within the range of those reported by others. However, it must be noted that a direct comparison of the kinetic values reported here and those from the literature should be viewed with caution as experimental protocols and genotypic characteristics of plant materials differ substantially from one lab to an-

Table 1. Significant effects in two-way analysis of variance (ANOVA) for data presented in Figure 2. Data from each site are analyzed separately

Source	NH ₄ ⁺			NO ₃ ⁻		
	df	MS	Fcalc	df	MS	Fcalc
Oak Ridge-Tree species						
Species	1	19.89	3.26	1	31.95	4.19
CO ₂	1	9.28	1.52	1	3.79	0.51
Sp* CO ₂	1	78.69	12.88**	1	2.36	0.31
Error	8	6.11		8	7.63	
Auburn-Crop species						
Species	1	167.90	3.00	1	38.30	1.95
CO ₂	1	1.30	0.02	1	5.90	0.30
Sp* CO ₂	1	1.00	0.02	1	45.70	2.33
Error	8	56.01		8	19.64	

*Significant at $P < 0.05$; ** Significant at $P < 0.01$.

other. Additionally, a direct comparison may also be difficult due to inconsistency in expression of uptake rate units. Nevertheless, in our study the mean V_{max} value for sugar maple, $21.4 \mu\text{mol g}^{-1} \text{h}^{-1}$, fell within the range of the values reported for the same species by Rothstein et al., 1996. In fact, V_{max} values reported for the tree species tested in the present study are in good agreement with the values generally reported for deciduous trees (Chapin et al., 1986; Gessler et al., 1998; Lajtha, 1994). Our kinetic results for crop species (Figure 2) are also within the range of values reported for soybean (Chaillou et al., 1994; Delhon et al., 1996; Saravitz et al., 1998), grasses (Goyal and Huffaker, 1986; Hole et al., 1990; Lee and Rudge, 1986; Lycklama, 1963; Macduff and Jackson, 1992; Siddiqi et al., 1989; Smart and Bloom, 1998) and other herbaceous species (Høgh-Jensen et al., 1997; Lainé et al., 1993; Macklon et al., 1990; Wieneke, 1992).

Our results are also in general agreement with other reports demonstrating a differential root uptake capacity for NH₄⁺ as opposed to NO₃⁻ in most native and crop species (Blacquiere, 1987; Haynes and Goh, 1978; Marschner et al., 1991). We found that all species tested here had a distinct preference for NH₄⁺ over NO₃⁻ but this preference was more pronounced for tree species compared to crop species (Figures 1 and 2). The greater preference for NH₄⁺ vs. NO₃⁻ is almost a universal root characteristics in tree species and is often associated with an adaptation to forest soils that

are relatively low in NO₃⁻ content (Cole, 1981; Haynes and Goh, 1978; Kronzucker et al., 1997; Marschner et al., 1991). Root preference for NH₄⁺ uptake is not limited to tree species since most crop species also prefer NH₄⁺ compared to NO₃⁻ (Glass and Siddiqi, 1995; Lee and Drew, 1989) when both N forms are equally present in the root medium roots.

There are a number of advantages in using the method described here as opposed to excised root techniques for determining root physiological uptake capacity for N. The most important advantage in this field method is that the target roots remain attached to the plant during uptake measurements. Therefore, unlike the excised root method, much of exchange of compounds between root and shoot remains uninterrupted. This is an important consideration particularly since the delivery of carbohydrates and nitrogenous compounds to the root and/or removal of absorbed N from the root play a critical role in net rates of NH₄⁺ and NO₃⁻ uptake (Glass and Siddiqi, 1995; Pate and Layzell, 1990; Saravits et al., 1998). Uptake kinetics measurements of intact roots as described here also has the advantage of not requiring major equipment or radioactive/stable isotope tracers at the field site.

Although we have successfully applied this method to assess kinetics of N uptake in both tree and crop species, its application may be subject to some limitations. These limitations have also been discussed by Rennenberg et al. (1996). For example, because

root excavation can damage fine roots, this procedure is most suitable for field situations where either digging is carried out with relative ease (e.g. coarse soils) or a substantial portion of the fine roots appear near the soil surface. In the present study both of these conditions were met but attempts are underway in our lab to improve the application of this method to more diverse soil conditions. It is also important to note that under field conditions most plant roots are mycorrhizal. Both endo- and ectomycorrhizae affect net N uptake rates (Boxman and Roelofs, 1988; Eltrop and Marschner, 1996; Rygielwicz et al., 1984) but at present this method does not separate the relative contribution of the mycorrhizae to overall N uptake. This problem is not unique to our method, but we suggest that until further quantification of root vs. mycorrhizal contribution, the term 'root system' rather than 'root' uptake kinetics be used.

CO₂ response

Because CO₂ enrichment generally increases carbohydrate levels in roots (BassiriRad et al., 1996a; Norby, 1994; Tschaplanski et al., 1993), metabolically regulated processes such as specific root N uptake rate are expected to be stimulated under high CO₂. In addition, because energy requirements associated with the uptake and assimilation of NO₃⁻ is considerably greater than NH₄⁺ (Blacquiére, 1987; Glass and Siddiqi, 1995; Haynes and Goh, 1978) we expected a greater positive effect of CO₂ on NO₃⁻ than NH₄⁺. However, with the exception of red maple, CO₂ did not significantly affect uptake kinetics of either N forms in species tested (Table 1 and Figure 2). Even though these results are inconsistent with our expectations they are not entirely surprising. Using potted seedlings we have shown elsewhere (BassiriRad et al., 1997a, b) that high CO₂ increased, decreased or had no significant effect on NO₃⁻ uptake kinetics depending upon species tested. In those studies the species dependent nature of the N uptake responses may have resulted from differences in patterns of C partitioning. If a greater proportion of the additional carbon resulting from growth under high CO₂ is partitioned into root growth then high CO₂ can still lead to an increase N uptake on a whole plant basis while uptake rates per unit root mass remains unchanged. In fact, in a different pot experiment, we found that CO₂ enrichment downregulated NH₄⁺ uptake capacity in two conifer seedlings, yet the whole plant N uptake increased in response to CO₂ (BassiriRad et al., 1996b).

In that case, increased biomass allocation to fine roots under high CO₂ counteracted the inhibitory effect of CO₂ on root N uptake capacity. Other mechanisms involved in regulation of root system uptake of N such as changes in root morphology, increased mycorrhizal hyphae and decreased plant demand by higher nitrogen use efficiency may be simultaneously operative in determining the magnitude and the direction of kinetic changes in response to CO₂. Finally, in most plants root kinetics of N uptake is regulated by demand which is likely to exhibit a seasonal pattern (Gessler et al., 1998; Glass and Siddiqi, 1995). Recently, in a hydroponic experiment using soybean and sunflower, we observed that root N uptake kinetics responses to CO₂ enrichment was highly dependent on the stages of development and root age (unpublished). Therefore, we suggest that a 'one point in time' determination is not adequate and more measurements of root N uptake kinetics are necessary to draw valid conclusions about possible effects of CO₂.

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